PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

ELZABURU, Alberto
Miguel Angel, 21
28010 Madrid
ESPAGNE

ELZABURU JL
2377402 - 13/05/2005

PCT

WRITTEN OPINION
(PCT Rule 66)

Date of mailing *(day/month/year)*	10.05.2005
Applicant's or agent's file reference PCT-154	**REPLY DUE within 0 month(s) and 10 days** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/ES2004/070001	21.01.2004	28.01.2003

International Patent Classification (IPC) or both national classification and IPC
C12N15/09

Applicant
EFARMES S.A. et al.

1. This written opinion is the **second** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☒	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis. For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 28.05.2005

Name and mailing address of the international preliminary examining authority:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer
Mossier, B

Formalities officer (incl. extension of time limits)
Brandt, M
Telephone No. +49 89 2399-2926

I. Basis of the opinion

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed")*:

Description, Pages

1-56, 58-61	as originally filed
57	filed with telefax on 19.04.2005

Sequence listings part of the description, Pages

1-91	as originally filed

Claims, Numbers

1-14	filed with telefax on 19.04.2005

Drawings, Sheets

1/3-3/3	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: English , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   ☐ the language of publication of the international application (under Rule 48.3(b)).
   ☒ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☒ furnished subsequently to this Authority in computer readable form.
   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with for the following reasons and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees:

   see separate sheet

3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1, 13
Inventive step (IS)	Claims	1-6, 13 and 14
Industrial applicability (IA)	Claims	

2. Citations and explanations

   see separate sheet

Exceptionally, in view of the telephone call of the 04.05.2005 a second written opinion is being issued. It should be noted that the next communication from the office will be the IPER.

Present application relates to *in vitro* methods for detecting individuals who are predisposed to the disease named Familial Hypercholesterolemia (FH), and more particularly to a method for detecting the presence or absence of several mutations associated with FH. 54 gene mutations and polymorphisms that are all produced in the gene sequence of the low density lipoprotein receptor gene (LDL-r) set forth in SEQ ID NO:1 are disclosed. Assay kits comprising oligonucleotides that hybridize with any of said mutations, methods of diagnosis characterised in that at least one of said 54 mutations is detected in a biological sample as well as oligonucleotides hybridising with any of the mutations comprised in said sequences are claimed.

Re Item I

Basis of the report

I.1 The amendments filed with the telefax dated 19.04.2005 appear to fulfill the requirements of Article 34(2)(b) PCT.

Re Item IV

Lack of unity of invention

The present application appears to lacks unity within the meaning of Rule 13.1 PCT. The following separate inventions can be considered:

a) Invention 1 (Claims 1 - 14; all partially):

Claims 1 - 14 relate to the mutation (-23)A> C in the LDL-r gene (SEQ ID NO:1). The subject-matter of said claims further encompasses sequences hybridizing with said mutation as well as methods for the detection of said mutation in a biological sample.

b) Inventions 2 - 54 (Claims 1 - 14; all partially):

As for Invention 1, but respectively relating to the mutations 1054del11, 108delC,.....[1587-5del15;1587del31] (i.e. Invention 2 corresponding to the mutation 1054del11; Invention 3 corresponding to the mutation 108delC.....; Invention 54 corresponding to the mutation [1587-5del15;1587del31]) and the subject matter relating to said mutation.

The 54 inventions are not so linked as to form a general inventive concept for the following reasons:
The problem to be solved by the present application can be seen as the detection of mutations in the LDL-r gene with the aim to use them in diagnosis of FH.
The solution to this problem is provided with the 54 mutations referred to in claim 1, respectively in claim 9.
However, this general concept is "*a priori*" not novel, since a number of LDL-r gene mutations in FH had been publicly known before the priority date of the present application (see ISR: e.g. D1: page 3, line 21 - page 5, line 13; D2: Table 1). Also, it had been publicly known before the priority date of the present application to detect lipid metabolic errors such as FH by noting these mutations.
Therefore and since no other technical feature can be distinguished which might link the subject matter of said claims, each of the above mentioned group of claims represents an independent invention.
Hence, the present application does not meet the requirements of unity of invention as defined in Rules 13.1 and 13.2 PCT.

Re Item V
Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

V.1 The following documents were taken into account:
D1: WO 02 06467 A1, respectively EP1304374
D2: FOUCHIER S. ET AL: 'The molecular basis of familial hypercholesterolemia in The Netherlands.' HUMAN GENETICS vol. 109, no. 6, December 2001, pages 602 - 615, XP002980736

The European Patent Application EP1304374 which has been published in accordance with Article 158(3) EPC corresponds to the PCT application WO0206467 published on the 24.01.2002. Hence, EP1304374 is validly considered as English translation of WO0206467.

V.2 Novelty (Article 33(1) and (2) PCT)

V2.1 The subject-matter of D1 relates to a method of detecting abnormalities or errors of lipid metabolism through employment of gene mutations, such as mutations in the LDL receptor gene, as indices. Several FH cases are described by D1 and FH case 4

was found to have a substitution T>A at nucleotide 283(T) in exon 3 of the LDL-r gene (see Figure 15 and page 43, lines 7 and 8). Due to the present wording of claim 13 (...*oligonucleotides* ***hybridizing*** *with*...) the polynucleotide sequence shown in Figure 15 is considered to be novelty destroying for the subject-matter referred to in claim 13 (see mutation C74Y). Moreover, since the term "assay kit" has no technical meaning D1 further anticipates the subject-matter referred to in claim 1. Hence, claims 1 and 13 appear to lack novelty under Article 33(2) PCT.

V2.2 With regard to the available prior art, the subject matter of claims 2 - 12 and 14 is considered as novel since it is not anticipated by the available prior art. Hence, it complies with the requirements of Article 33(1) and (2) PCT.

V.3 Inventive Step (Article 33(1) and (3) PCT)

V3.1 The technical contribution of claims 1 - 6, 13 and 14 can be summed up as the provision of 54 mutations found in the LDL-r gene as referred to in claim 1.
With regard to prior art (D1 and D2 already disclose mutations in the LDL-r gene and methods for the detection of the same; D1: page 3, line 21 - page 5, line 13; D2: Table 1), the technical problem solved by the present application is the provision of alternative mutations to be found in the LDL-r gene.
The 54 mutations as referred to in claim 1 are found out merely by comparing the base sequence of the normal LDL-r gene with the base sequences of LDL-r genes of patients clinically diagnosed as suffering form FH. Hence, taking into consideration the prior art in combination with general knowledge, the provision of further gene sequences comprising mutations in the LDL-r gene would be obvious and straight forward for the person skilled in the art.
Moreover, the IPEA raises the Applicant's attention to the fact that with regard to the statement of D2, namely that FH patients from different populations are characterised by different mutations in the LDL-r gene (D2: page 611, column 2, paragraph 2 - page 612, column 1, paragraph 4), it could be further expected that screening of Spanish FH cases would reveal different mutations compared to the ones obtained by screening Dutch (D2) and/or Japanish FH samples (D1).
Thus, it is not possible to acknowledge inventive step for the subject-matter referred to in claims 1 - 6, 13 and 14 and said claims are therefore considered to lack inventive step under Article 33(3) PCT.

V3.2 Claims 7 and 8 relate to the use of oligonucleotides in extracorporeal methods of in vitro detection of the LDL-r gene mutations for diagnosis of FH and the subject-matter fo claims 9 - 14 relate to extracorporeal methods of diagnosis of FH characterized in that in a biological sample at least one mutation in the LDL-r gene as referred to is detected.
D1 that can be considered as closest prior art for evaluating the inventiveness of the subject-matter referred to in claims 7 - 14 discloses methods for the detection of mutations in the LDL-r gene (e.g.: page 3, line 21 - page 5, line 13).
Present application differs from D1 in that the method is characterised by mutations that were found in the Spanish population whereas D1 relates to methods that are based on mutations to be found in the Japanese population. Hence, the technical problem to be solved can be seen as the provision of a method to diagnose FH in the Spanish population.
Since the available prior art neither discloses nor suggests a method for the diagnosis of FH caused by different mutations in the Spanish population and since apparently the claimed method enables a very specific system for the diagnosis of FH in Spain, the subject-matter referred to in claims 7 - 14 appears to be inventive (Article 33(3) PCT).

Certain Observations on the International Application

*The following remarks on **Clarity and Sufficiency of Disclosure** (Article 6 and 5 PCT) are made:*
*1) Claims 1, 13 and 14 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined (...oligonucleotides **hybridising....**). The claims attempt to define the subject-matter in terms of the result to be achieved, which merely amounts to a statement of the underlying problem, without providing the technical features necessary for achieving this result. Moreover, the term "hybridising", without the indication of the hybridisation conditions, is vague and unclear and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claims unclear, Article 6 PCT.*

2) In order to avoid any ambiguities with regard the "positions of the different mutations" the LDL-r gene has to be characterised by a specific SEQ ID NO (Article 6 PCT).